

## **Supplemental Information**

### **Autophagy and Lipid Metabolism Coordinately Modulate Life Span in Germline-less *C. elegans***

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#### **Supplemental Inventory**

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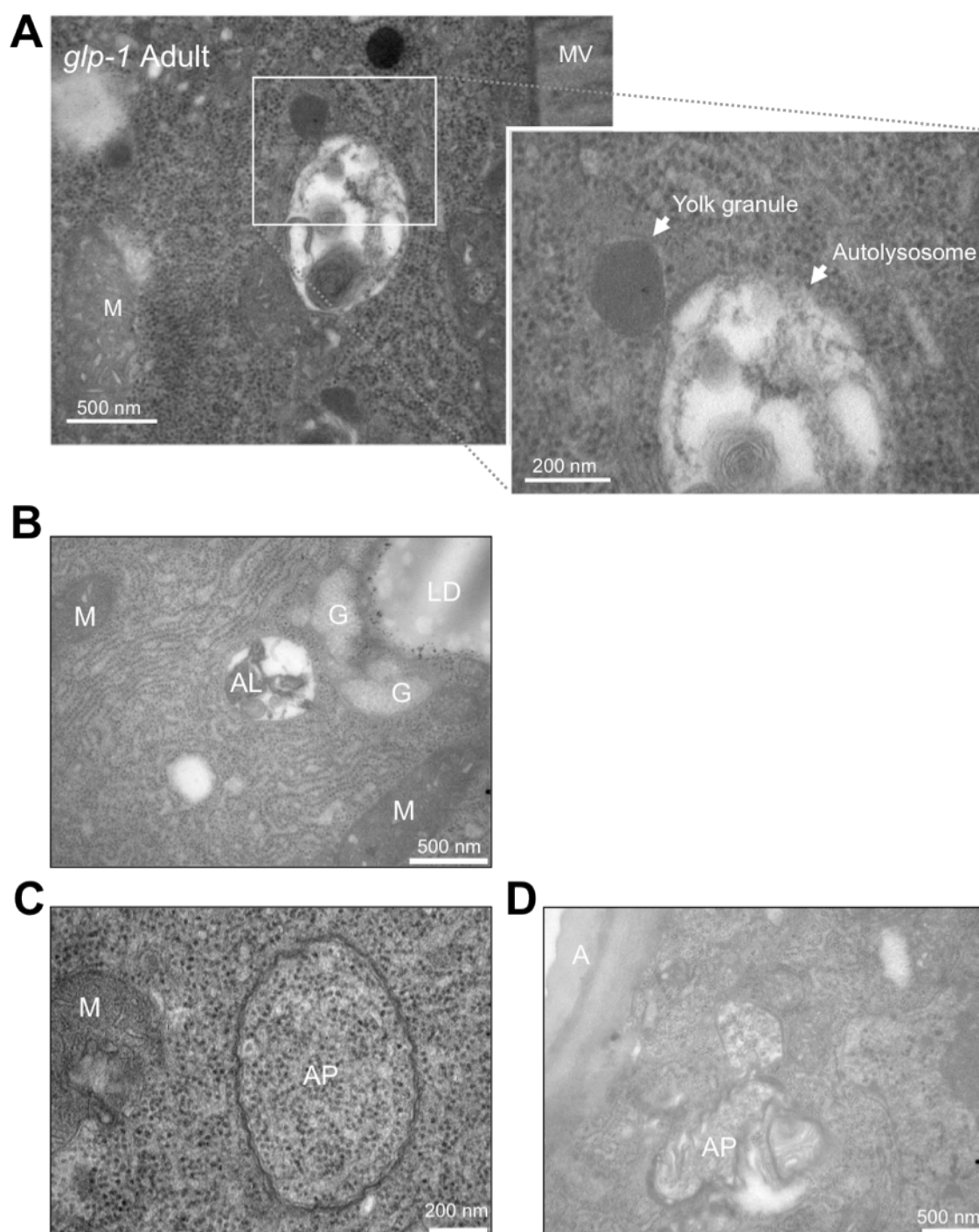
Table S3, related to Figure S2I and S5F

##### **2. Supplemental Experimental Procedures**

##### **3. Supplemental References**

Figure S1

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**Fig. S1. Electron Micrographs of Autophagic Events in *glp-1* animals, related to Figures 1A and 1B**

(A) Representative electron micrograph of an intestinal cell in a 1-day old *glp-1(e2141)* animal raised at the restrictive temperature (details of micrograph shown in Fig. 1A). A yolk granule appears to be about to fuse with an autolysosome containing disintegrating cytoplasmic components, including several secondary double-membranes. Single-membrane autolysosomes and double-membrane autophagosomes were observed frequently in the *glp-1* animals in the intestine as well as in hypodermal seam cells (see Fig. S1D) compared to wild-type animals. The size and contents of these autophagic events were variable, and it was only in this micrograph that we observed an autolysosome to associate with a yolk granule. MV, microvilli, M, mitochondria.

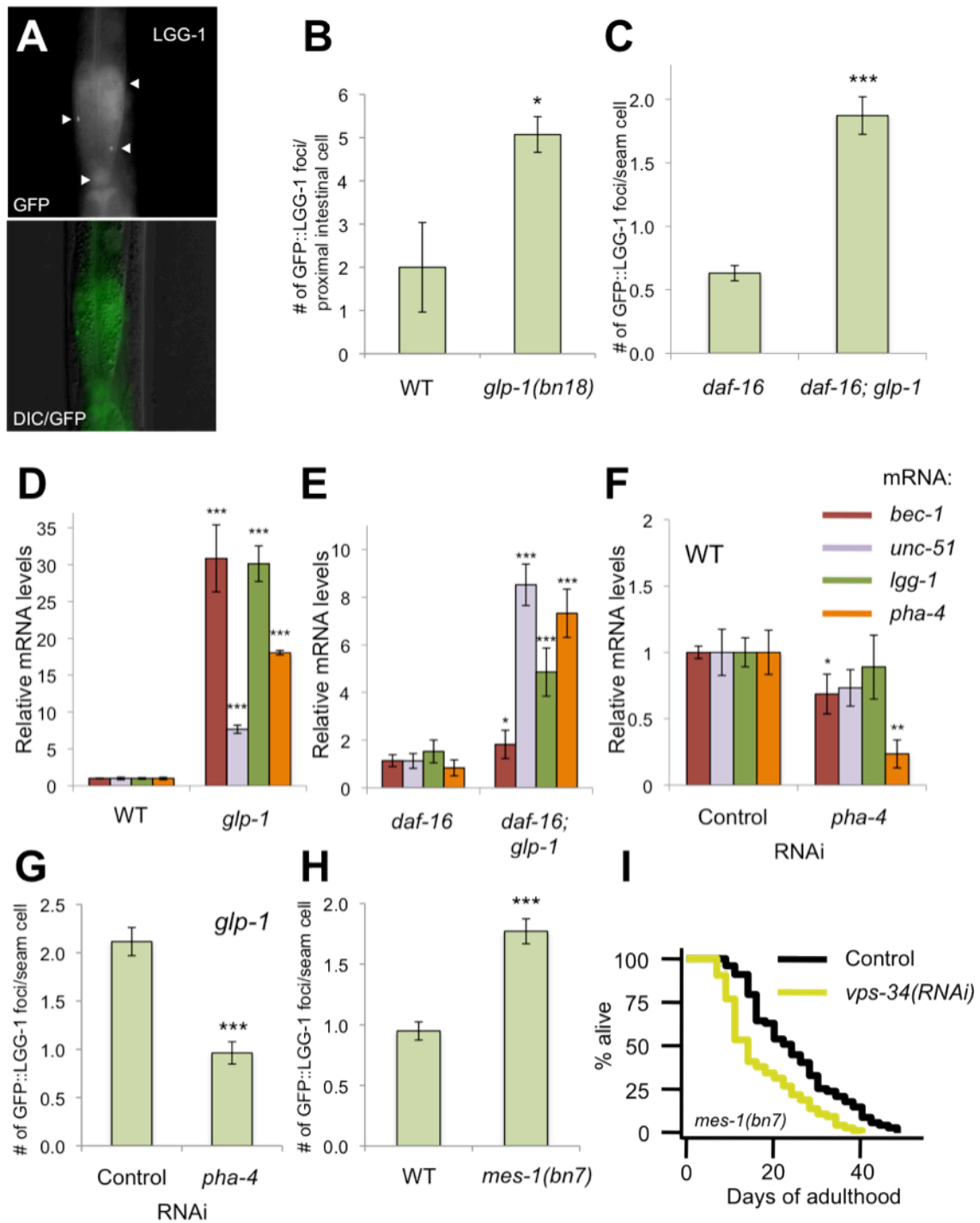
(B) Additional micrograph showing autolysosome with membrane-like structures undergoing degradation (AL), as well as a lipid droplet (LD), glycogen (G) and mitochondria (M) in an intestinal cell of a 1-day old *glp-1(e2141)* animal.

(C) Micrograph of intestinal cell with double membrane-bound autophagosome (AP) containing rough ER with adjacent mitochondria (M).

(D) Micrograph of hypodermal seam cell containing a complex autophagosomal structure (AP) with multiple membranes partially surrounding it. 'A' highlights the alae of the seam cell.

Figure S2

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**Fig. S2. Analysis of Autophagy Phenotypes in Germline-less Animals, related to Figures 1C-1F and Figure 2**

(A) Representative micrograph shown either as GFP (top) or GFP with DIC overlay (bottom, pseudo-colored) of the proximal part of the intestine of *glp-1(e2141)* animals expressing GFP::LGG-1. To quantify autophagy in intestinal cells, GFP-positive foci (i.e., punctate structures, arrowheads) were counted in the first three intestinal cells, beyond the pharynx. See also Supplementary Materials and Methods.

(B) Quantification of GFP::LGG-1 foci in wild-type N2 (WT) and in *glp-1(bn18)* intestinal cells. Animals were raised at the non-permissive temperature from hatching. Cumulative counts of two experiments are shown (n=15 animals) as mean  $\pm$  SEM (\* $P$  < 0.05,  $t$ -test).

(C) Quantification of GFP::LGG-1-positive foci in seam cells of 1-day old adult *daf-16(mu86)* and *daf-16(mu86); glp-1(e2141)* animals. Animals were raised at the non-permissive temperature (25°C) from hatching and mean number of foci  $\pm$  SEM is shown. This experiment was counted at the same time as experiment shown in Fig. 1D (n=100-250 cells, \*\*\* $P$  < 0.001 vs. WT in Fig. 1D, ANOVA).

(D) RT-PCR analysis of mRNA levels of autophagy genes (*bec-1*, *unc-51*, and *lgg-1*) and of *pha-4* in 3-day old adult N2 wild-type (WT) and *glp-1(e2141)* animals. Normalized data of biological triplicates are shown as mean  $\pm$  SD. \*\*\* $P$  < 0.001 vs. WT, ANOVA.

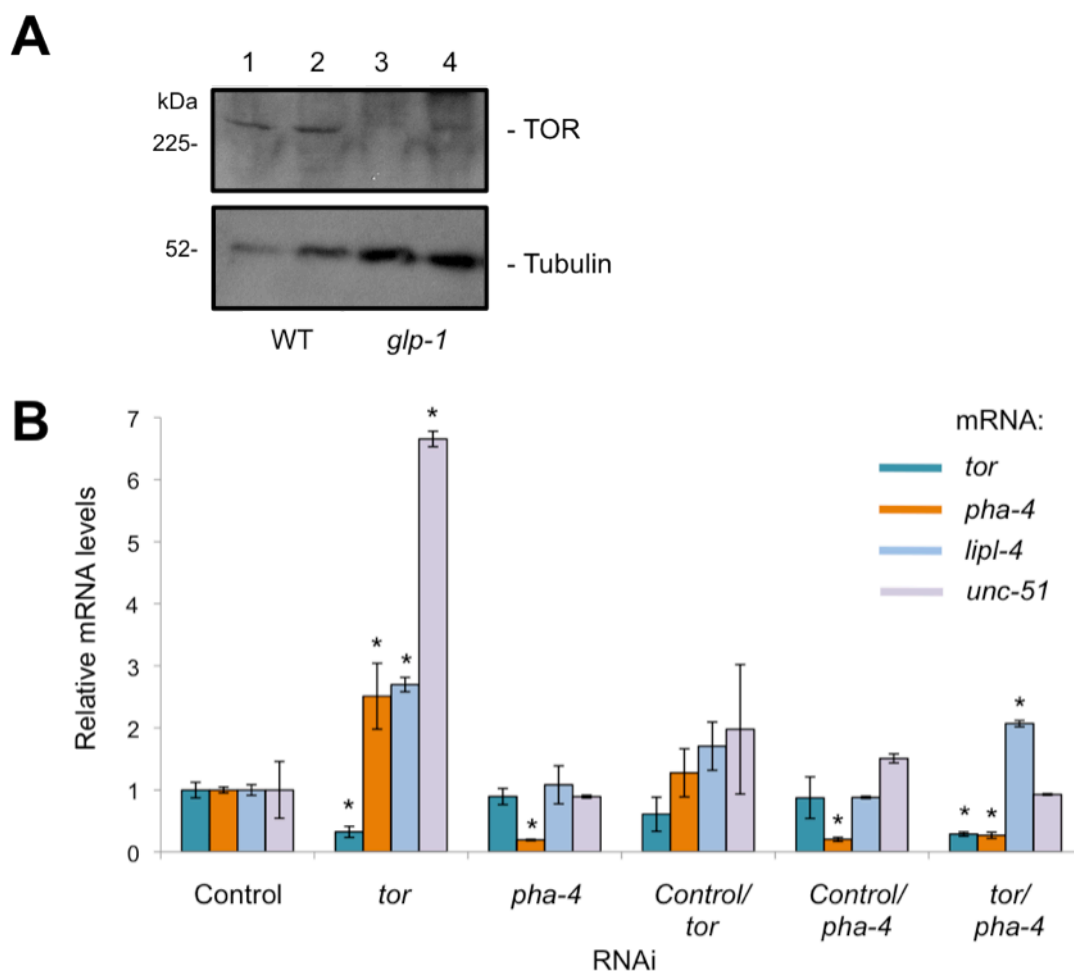
(E) RT-PCR analysis of mRNA levels of autophagy genes (*bec-1*, *unc-51*, and *lgg-1*) and of *pha-4* in 1-day old adult *daf-16(mu86)* and *daf-16(mu86); glp-1(e2141)* animals. This experiment was performed at the same time as experiment shown in Fig. 1E. Relative mean expression  $\pm$  SD is shown (\* $P$  < 0.05, \*\*\* $P$  < 0.001 vs. WT in Fig. 1E, ANOVA).

(F) RT-PCR analysis of mRNA levels of autophagy genes (*bec-1*, *unc-51*, and *lgg-1*) and of *pha-4* in wild-type worms fed control bacteria or bacteria expressing *pha-4* dsRNA from Day 1 to Day 3 of adulthood. Normalized data of biological triplicates are shown as mean  $\pm$  SD (\* $P$  < 0.05, \*\* $P$  < 0.01 vs. control, ANOVA).

(G) Quantification of GFP::LGG-1 foci in *glp-1(e2141)* larvae (L3 larva stage) fed control bacteria or bacteria expressing dsRNA for the FOXA transcription factor *pha-4* for one generation. These animals were raised at 25°C from hatching to prevent germline development. Cumulative counts of two independent experiments (n=115-130 seam cells). Data are shown as mean  $\pm$  SEM (\* $P$  < 0.05; \*\* $P$  < 0.01 vs. control,  $t$ -test).

(H) Quantification of GFP::LGG-1 foci in day 1 wild-type N2 (WT) and *mes-1(bn7)* animals. Non-sterile *mes-1* animals showed autophagy levels similar to wild-type animals (data not shown). Animals were raised at 25°C from hatching for 2 days; this treatment induces ~50% sterile, long-lived *mes-1* animals (Arantes-Oliveira et al., 2002). Cumulative counts of at least three independent experiments are shown (n~140 seam cells) as mean  $\pm$  SEM (\*\*\* $P$  < 0.001 vs. WT,  $t$ -test).

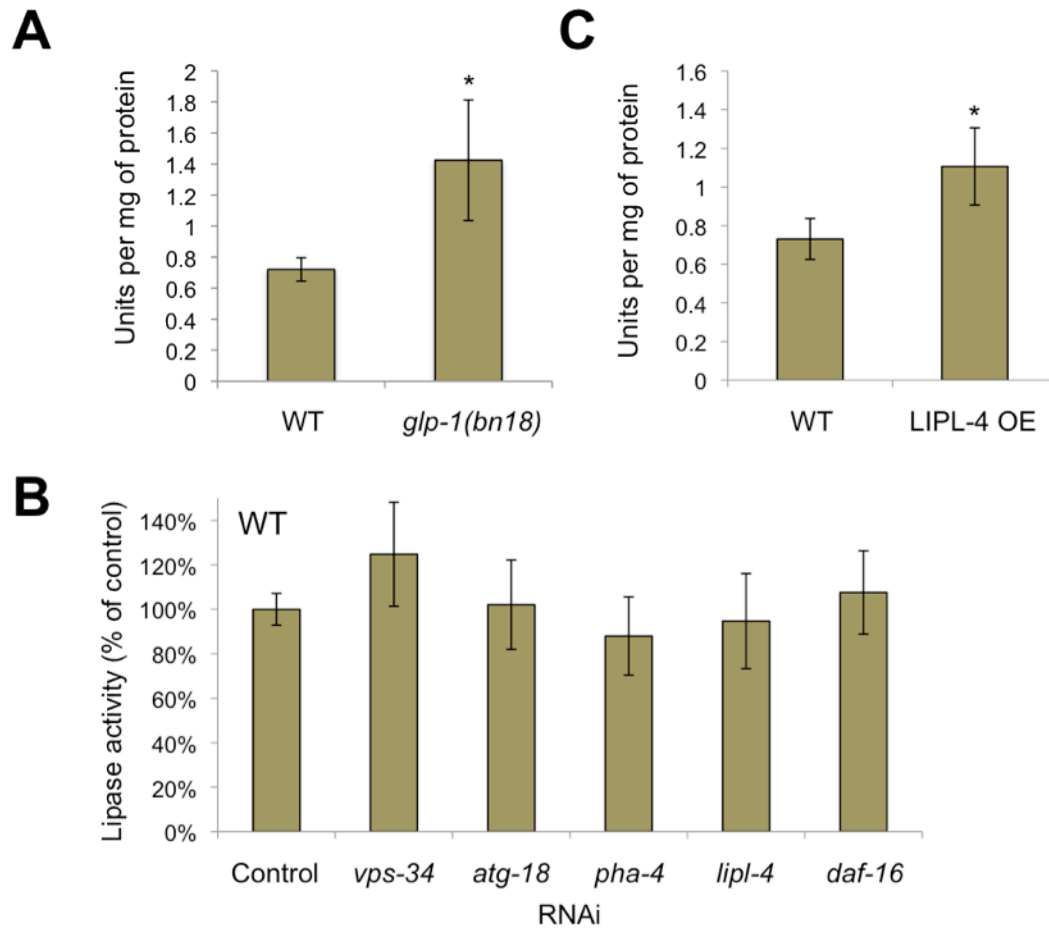
(I) Lifespan analysis of sterile *mes-1(bn7)* animals raised at 25°C and moved to 20°C onto control bacteria or bacteria expressing dsRNA against *vps-34*. See Table S3 for details and additional experiments of other autophagy-related genes.



**Fig. S3. TOR Levels in *glp-1* Animals, and TOR-regulated Gene Expression in Wild-type animals, related to Figure 3**

**(A)** Immunoblot of TOR and tubulin loading control of 1-day old adult N2 wild-type (WT) (lane 1 and 2) and *glp-1(e2141)* (lane 3 and 4) animals (representative result of two separate experiments).

**(B)** 1-day old N2 wild-type adult worms were fed control bacteria or bacteria expressing *tor* dsRNA, *pha-4* dsRNA, or a 1:1 bacterial mix (Control/*tor*, Control/*pha-4* and *tor/pha-4*) for 48 hours and mRNA levels of *tor*, *pha-4*, *lipi-4*, and *unc-51* were measured using RT-PCR. Data are shown as mean  $\pm$  SD (biological duplicates; \* $P < 0.05$  vs. control, ANOVA). While a previous report observed no changes in PHA-4 protein levels in the pharynx following feeding with a mixture of *tor* and *daf-15* RNAi (Sheaffer et al., 2008), our data suggests that TOR can regulate *pha-4* expression at the transcriptional level.



**Fig. S4. Lipase Activity Measurements in *glp-1(bn18)* and LIPL-4 Overexpressing Animals, related to Figure 4**

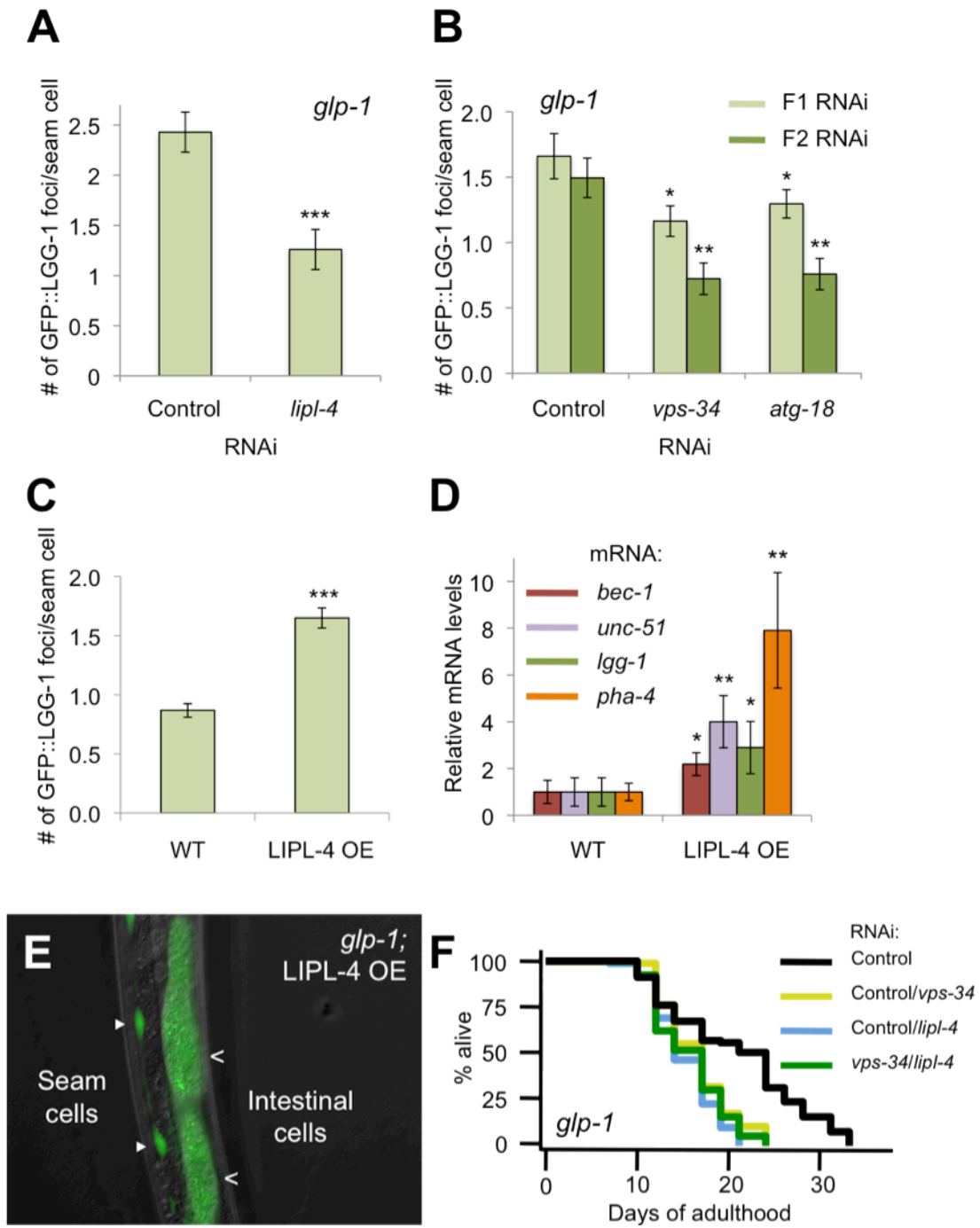
(A) Lipase activity was measured in 1-day old *glp-1(bn18)* animals or in non-transgenic siblings (WT). Worms were raised at 25°C from hatching to prevent germline development (n=3 biological replicates; \* $P < 0.05$  vs. WT,  $t$ -test).

(B) 1-day old adult N2 wild-type (WT) animals were subjected to dsRNA against autophagy genes, *pha-4*, *lipl-4*, or *daf-16* for 3 days and lipase activity was measured (n=3-6 biological replicates). None of the RNAi treatments resulted in statistically significant changes in lipase activity.

(C) Lipase activity was measured by a colorimetric assay in 1-day old animals overexpressing LIPL-4/K08A4.5 (LIPL-4 OE) (Wang *et al.*, 2008), or in non-transgenic siblings (WT). Data of biological triplicates are shown as mean  $\pm$  SD (\* $P < 0.05$ ,  $t$ -test).

Figure S5

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**Fig. S5. Characterization of LIPL-4 Function in Autophagy, related to Figure 5**

(A) Quantification of GFP::LGG-1-positive foci in seam cells of *glp-1(e2141)* larvae (L3 larva stage) fed control bacteria or bacteria expressing dsRNA against *lipl-4* for three “F2” generations. Cumulative counts of two independent experiments (n~60 seam cells). Data are shown as mean  $\pm$  SEM ( $***P < 0.001$  vs. control, *t*-test).

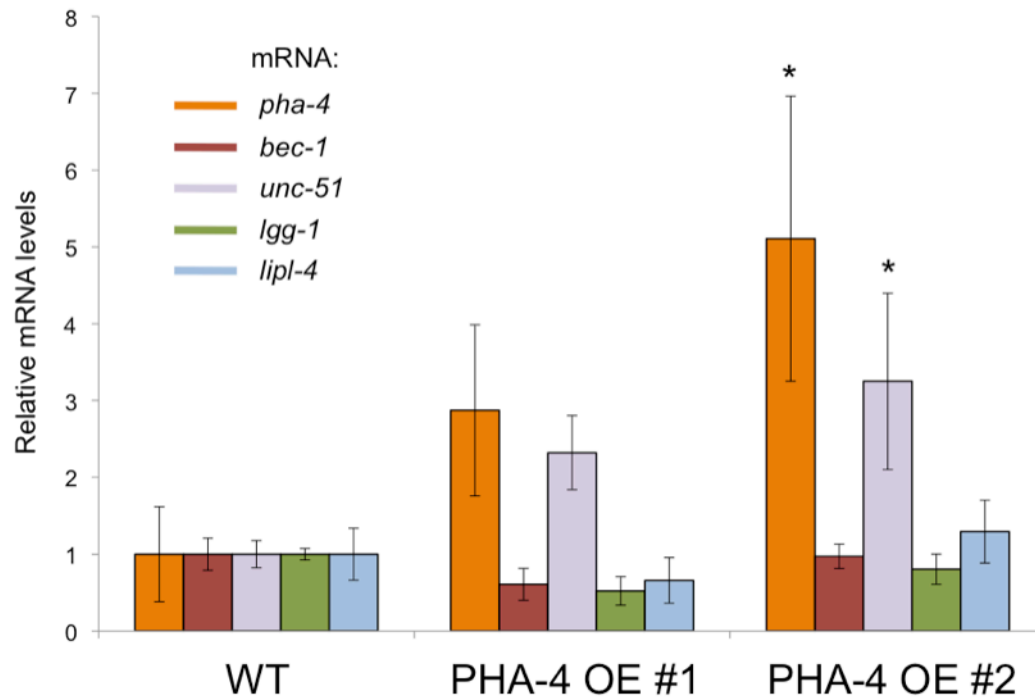
(B) Quantification of GFP::LGG-1 foci in *glp-1(e2141)* larvae (L3 larva stage) fed control bacteria or bacteria expressing dsRNA for autophagy genes *vps-34* or *atg-18* for two (“F1”) or three (“F2”) generations. At each generation analyzed, worms were raised at 25°C from hatching to prevent germline development. Cumulative counts of two independent experiments (n=80-115 seam cells). Data are shown as mean  $\pm$  SEM ( $*P < 0.05$ ,  $**P < 0.01$ , ANOVA).

(C) Quantification of GFP::LGG-1-positive foci in seam cells of animals overexpressing LIPL-4 (LIPL-4 OE) L3 larvae and in non-transgenic siblings (WT). Animals were raised at 20°C, and mean number of foci  $\pm$  SEM is shown (n~280 seam cells,  $***P < 0.001$ , *t*-test).

(D) RT-PCR analysis of *bec-1*, *unc-51*, *lgg-1*, and *pha-4* mRNA in 1-day adult WT and LIPL-4 OE animals. Animals were raised at 20°C, and mean expression  $\pm$  SD is shown ( $*P < 0.05$ ,  $**P < 0.01$ , ANOVA).

(E) Representative picture of a day 1 adult transgenic *glp-1(e2141)* animals expressing LIPL-4 raised at 25°C. LIPL-4 expression was visualized by GFP co-expression from a polycistronic transcriptional unit (Wang et al., 2008). LIPL-4 expression was seen in the intestinal cells (arrowheads) and hypodermal seam cells (filled arrows), and the expression patterns were similar but of lower magnitude, in *glp-1(+)* animals (data not shown, see also Supplemental Information). Magnification, 1000-fold.

(F) Lifespan analysis of *glp-1(e2141)* animals fed control bacteria or a 1:1 ratio mix of control bacteria with bacteria expressing *vps-34* or *lipl-4* dsRNA, or a mix of *vps-34* and *lipl-4* RNAi bacteria. Experiments were performed twice with similar results; see Table S3 for details and repeats. Animals were raised at 25°C from hatching until the first day of adulthood and were then moved to 20°C for the remainder of their life.



**Fig. S6. Gene Expression Analysis in PHA-4 Overexpressing Animals, related to Figure 6**

RT-PCR analysis of mRNA levels of *pha-4*, autophagy genes (*bec-1*, *unc-51*, and *lgg-1*) and of *lipi-4* were measured in 1-day old N2 wild-type animals (WT) or in two different strains overexpressing PHA-4, PHA-4 OE #1 (Zhong *et al.*, 2010) and PHA-4 OE #2 (Panowski *et al.*, 2007). Strains were raised at 20°C. Normalized data of biological triplicates are shown as mean expression  $\pm$  SD is shown (\* $P < 0.05$  vs. WT, ANOVA). We note that the PHA-4 OE #2 strain has not been outcrossed to the WT strain.

Table S1

| STRAINS      | Adult-only RNAi treatment | Target RNAi avg lifespan (days) | #of RNAi animals | Control RNAi avg lifespan (days) | #of RNAi animals | $\Delta$ avg (%) | P value vs control |
|--------------|---------------------------|---------------------------------|------------------|----------------------------------|------------------|------------------|--------------------|
| WT           | <i>pha-4*</i>             | 17.4                            | 93/100           | 18.7                             | 61/100           | -7               | 0.017              |
|              | <i>pha-4</i>              | 18.4                            | 88/100           | 18.8                             | 64/100           | -2               | 0.51               |
|              | <i>pha-4</i>              | 18.6                            | 91/100           | 20.2                             | 65/100           | -8               | 0.0004             |
|              | <i>vps-34</i>             | 19.3                            | 78/100           | 18.3                             | 83/100           | 6                | 0.19               |
|              | <i>vps-34</i>             | 17.5                            | 68/100           | 17.9                             | 67/100           | -2               | 0.27               |
|              | <i>vps-34*</i>            | 18.2                            | 79/100           | 19.2                             | 61/100           | -5               | 0.52               |
|              | <i>bec-1</i>              | 19.6                            | 82/100           | 18.3                             | 83/100           | 7                | 0.14               |
|              | <i>bec-1</i>              | 17.9                            | 71/100           | 17.9                             | 67/100           | 0                | 0.94               |
|              | <i>bec-1*</i>             | 19.2                            | 77/100           | 19.2                             | 61/100           | 7                | 0.27               |
|              | <i>lgg-1</i>              | 18.0                            | 68/100           | 17.9                             | 67/100           | 1                | 0.89               |
|              | <i>lgg-1</i>              | 20.2                            | 83/100           | 18.5                             | 61/100           | 9                | 0.06               |
|              | <i>lgg-1*</i>             | 18.8                            | 88/100           | 19.2                             | 61/100           | -2               | 0.30               |
|              | <i>unc-51*</i>            | 18.7                            | 72/100           | 18.3                             | 83/100           | 2                | 0.90               |
|              | <i>unc-51</i>             | 22.4                            | 65/100           | 20.4                             | 58/100           | 10               | 0.068              |
|              | <i>unc-51</i>             | 18.2                            | 51/60            | 18.6                             | 48/60            | -2               | 0.67               |
|              | <i>atg-18*</i>            | 19.3                            | 73/100           | 18.3                             | 83/100           | 6                | 0.38               |
|              | <i>atg-18</i>             | 19.7                            | 51/100           | 20.4                             | 58/100           | -3               | 0.52               |
|              | <i>atg-18</i>             | 18.8                            | 46/60            | 18.6                             | 48/60            | 10               | 0.85               |
|              | <i>tor*</i>               | 22.7                            | 68/100           | 17.9                             | 67/100           | 27               | <0.0001            |
|              | <i>tor</i>                | 22.7                            | 73/100           | 18.5                             | 61/100           | 23               | <0.0001            |
|              | <i>tor</i>                | 21.6                            | 75/100           | 19.2                             | 61/100           | 13               | 0.0046             |
|              | <i>tor</i>                | 22.4                            | 76/100           | 19.7                             | 62/100           | 14               | 0.0010             |
| <i>glp-1</i> | <i>pha-4*</i>             | 16.7                            | 92/100           | 22.3                             | 74/100           | -25              | <0.0001            |
|              | <i>pha-4</i>              | 17.0                            | 83/100           | 22.6                             | 81/100           | -25              | <0.0001            |
|              | <i>pha-4</i>              | 17.7                            | 90/100           | 21.7                             | 76/100           | -18              | <0.0001            |
|              | <i>vps-34</i>             | 16.4                            | 81/100           | 21.9                             | 71/100           | -25              | <0.0001            |
|              | <i>vps-34</i>             | 17.8                            | 95/100           | 24.0                             | 83/100           | -26              | <0.0001            |
|              | <i>vps-34*</i>            | 15.5                            | 85/100           | 20.3                             | 80/100           | -24              | <0.0001            |
|              | <i>bec-1</i>              | 17.4                            | 74/100           | 21.9                             | 71/100           | -22              | <0.0001            |
|              | <i>bec-1</i>              | 20.0                            | 97/100           | 24.0                             | 83/100           | -17              | 0.0005             |
|              | <i>bec-1*</i>             | 15.8                            | 79/100           | 20.3                             | 80/100           | -21              | <0.0001            |
|              | <i>lgg-1</i>              | 19.6                            | 95/100           | 24.0                             | 83/100           | -18              | 0.009              |
|              | <i>lgg-1</i>              | 19.9                            | 91/100           | 24.2                             | 84/100           | -18              | <0.0001            |
|              | <i>lgg-1*</i>             | 12.3                            | 90/100           | 20.3                             | 80/100           | -39              | <0.0001            |
|              | <i>unc-51*</i>            | 16.0                            | 75/100           | 21.9                             | 71/100           | -27              | <0.0001            |
|              | <i>unc-51</i>             | 21.5                            | 73/100           | 24.2                             | 69/100           | -11              | 0.040              |
|              | <i>unc-51</i>             | 19.6                            | 54/60            | 23.6                             | 53/60            | -17              | 0.0028             |
|              | <i>atg-18*</i>            | 14.3                            | 77/100           | 21.9                             | 71/100           | -35              | <0.0001            |
|              | <i>atg-18</i>             | 16.2                            | 58/100           | 24.2                             | 69/100           | -33              | <0.0001            |
|              | <i>atg-18</i>             | 17.1                            | 56/60            | 23.6                             | 53/60            | -28              | <0.0001            |
|              | <i>tor*</i>               | 21.9                            | 93/100           | 24.0                             | 83/100           | -9               | 0.056              |
|              | <i>tor</i>                | 23.9                            | 87/100           | 24.2                             | 84/100           | -1               | 0.72               |
|              | <i>tor</i>                | 18.5                            | 87/100           | 20.3                             | 80/100           | -9               | 0.16               |
|              | <i>tor</i>                | 22.3                            | 69/100           | 21.7                             | 72/100           | 3                | 0.60               |

**Table S1.** Lifespan analysis of N2 wild-type (WT) or germline-less *glp-1(e2141)* animals fed control bacteria or bacteria expressing dsRNA against *pha-4*, different autophagy genes or *tor*. The table is organized so lifespan experiments carried out in parallel in WT and *glp-1* animals are in the same order. Data shown includes average of mean lifespan (avg lifespan), observed events (# of RNAi animals), % mean lifespan difference vs control ( $\Delta$  avg) and *P* value vs control. *P* values calculated with Log-rank test (\*survival curves shown in Fig. 2A, 2B, 2C and 3B. These representative curves were randomly selected from larger lifespan sets). In all experiments, animals were raised at 25°C from hatching until the first day of adulthood and were then moved to 20°C for the remainder of their lives.

**Table S2**

| STRAINS   | Adult-only RNAi treatment | Target RNAi avg lifespan (days) | #of RNAi animals | Control RNAi avg lifespan (days) | #of RNAi animals | $\Delta$ avg (%) | P value vs control |
|-----------|---------------------------|---------------------------------|------------------|----------------------------------|------------------|------------------|--------------------|
| WT        | <i>bec-1</i>              | 20.2                            | 71/100           | 19.2                             | 59/100           | 5                | 0.10               |
|           | <i>bec-1</i>              | 19.2                            | 85/100           | 18.4                             | 80/100           | 4                | 0.77               |
|           | <i>bec-1*</i>             | 18.3                            | 83/100           | 18.5                             | 74/100           | -1               | 0.63               |
|           | <i>lgg-1</i>              | 17.8                            | 42/60            | 18.0                             | 43/60            | -1               | 0.71               |
|           | <i>lgg-1</i>              | 19.8                            | 87/100           | 18.4                             | 80/100           | 8                | 0.010              |
|           | <i>lgg-1*</i>             | 18.3                            | 83/100           | 18.5                             | 74/100           | -1               | 0.87               |
|           | <i>pha-4</i>              | 18.2                            | 79/100           | 19.0                             | 73/100           | -4               | 0.11               |
|           | <i>pha-4*</i>             | 18.7                            | 89/100           | 18.4                             | 80/100           | 2                | 0.58               |
|           | <i>pha-4</i>              | 17.4                            | 78/100           | 18.5                             | 74/100           | -6               | 0.064              |
|           | <i>vps-34</i>             | 20.3                            | 56/100           | 19.0                             | 73/100           | 7                | 0.080              |
|           | <i>vps-34</i>             | 19.0                            | 65/100           | 19.2                             | 59/100           | -1               | 0.58               |
|           | <i>vps-34*</i>            | 19.6                            | 82/100           | 18.4                             | 80/100           | 7                | 0.21               |
| LIPL-4 OE | <i>tor</i>                | 22.7                            | 60/100           | 19.0                             | 73/100           | 20               | <0.0001            |
|           | <i>tor</i>                | 23.1                            | 82/100           | 18.4                             | 80/100           | 26               | <0.0001            |
|           | <i>tor</i>                | 21.3                            | 64/100           | 18.5                             | 74/100           | 15               | 0.0011             |
|           | <i>bec-1</i>              | 20.4                            | 77/100           | 24.0                             | 53/100           | -15              | 0.0001             |
|           | <i>bec-1</i>              | 17.2                            | 68/100           | 22.7                             | 74/100           | -24              | <0.0001            |
|           | <i>bec-1*</i>             | 18.6                            | 80/100           | 21.4                             | 73/100           | -13              | 0.0001             |
|           | <i>lgg-1</i>              | 15.8                            | 37/50            | 19.2                             | 24/50            | -18              | 0.0026             |
|           | <i>lgg-1</i>              | 16.7                            | 64/100           | 22.7                             | 74/100           | -26              | <0.0001            |
|           | <i>lgg-1*</i>             | 18.5                            | 89/100           | 21.4                             | 73/100           | -14              | 0.0001             |
|           | <i>pha-4</i>              | 18.9                            | 74/100           | 23.5                             | 60/100           | -20              | <0.0001            |
|           | <i>pha-4*</i>             | 17.5                            | 81/100           | 22.7                             | 74/100           | -23              | <0.0001            |
|           | <i>pha-4</i>              | 16.4                            | 70/100           | 21.4                             | 73/100           | -23              | <0.0001            |
| LIPL-4 OE | <i>vps-34</i>             | 17.5                            | 64/100           | 23.5                             | 60/100           | -26              | <0.0001            |
|           | <i>vps-34</i>             | 20.7                            | 81/100           | 24.0                             | 53/100           | -14              | 0.0001             |
|           | <i>vps-34*</i>            | 16.7                            | 64/100           | 22.7                             | 74/100           | -27              | <0.0001            |
|           | <i>tor</i>                | 24.9                            | 77/100           | 23.5                             | 60/100           | 6                | 0.15               |
|           | <i>tor</i>                | 24.0                            | 77/100           | 22.7                             | 74/100           | 6                | 0.038              |
|           | <i>tor</i>                | 21.5                            | 80/100           | 21.4                             | 73/100           | 1                | 0.62               |

**Table S2.** Lifespan of transgenic animals overexpressing LIPL-4 (LIPL-4 OE) (Wang *et al.*, 2008) and non-transgenic siblings (WT) fed control bacteria or bacteria expressing dsRNA against *pha-4* or different autophagy genes. PHA-4 is unlikely to regulate *lipl-4* expression in these experiments, as *pha-4* RNAi did not affect the expression level of LIPL-4 in LIPL-4 overexpressing animals at least until Day 9 (data not shown). The table is organized so lifespan experiments carried out in parallel in WT and LIPL-4 OE animals are in the same order. We generally observed LIPL-4 OE to be ~20% longer-lived than WT on control RNAi (and Dr. M. Wang, pers. comm.). Data shown includes average of mean lifespan (avg lifespan), observed events (# of RNAi animals), % mean lifespan difference vs. control ( $\Delta$  avg), and *P* value vs. control. *P* values calculated with Log-rank test (\*survival curves shown in Figures 5A, and 5B. These representative curves were randomly selected from larger lifespan sets). Animals were incubated 20°C throughout their lives.

**Table S3**

| STRAINS             | Adult-only RNAi treatment | Target RNAi avg lifespan (days) | #of RNAi animals | Control RNAi avg lifespan (days) | #of RNAi animals | $\Delta$ avg (%) | P value vs control |
|---------------------|---------------------------|---------------------------------|------------------|----------------------------------|------------------|------------------|--------------------|
| <b>Fig. S2I</b>     |                           |                                 |                  |                                  |                  |                  |                    |
| <b>WT</b>           | <i>vps-34*</i>            | 16.9                            | 44/100           | 16.5                             | 49/100           | 2                | 0.96               |
|                     | <i>atg-18</i>             | 16.1                            | 60/100           | 16.6                             | 78/100           | 3                | 0.60               |
|                     | <i>pha-4</i>              | 14.0                            | 88/100           | 14.5                             | 70/100           | -3               | 0.50               |
| <b><i>mes-1</i></b> | <i>vps-34*</i>            | 17.1                            | 67/100           | 24.8                             | 69/100           | -31              | <0.0001            |
|                     | <i>atg-18</i>             | 12.2                            | 94/100           | 17.5                             | 75/100           | -30              | <0.0001            |
|                     | <i>pha-4</i>              | 10.4                            | 87/100           | 15.5                             | 85/100           | -30              | <0.0001            |
| <b>Fig. S5F</b>     |                           |                                 |                  |                                  |                  |                  |                    |
| <b><i>glp-1</i></b> | CTL/ <i>vps-34</i>        | 17.5                            | 83/100           | 23.0                             | 78/100           | -24              | <0.0001            |
|                     | CTL/ <i>lipI-4</i>        | 19.1                            | 82/100           | 23.0                             | 78/100           | -17              | 0.0019             |
|                     | <i>vps-34/lipI-4</i>      | 19.2                            | 80/100           | 23.0                             | 78/100           | -17              | <0.0001            |
|                     | CTL/ <i>vps-34*</i>       | 16.5                            | 58/100           | 20.9                             | 72/100           | -21              | <0.0001            |
|                     | CTL/ <i>lipI-4*</i>       | 15.3                            | 72/100           | 20.9                             | 72/100           | -27              | <0.0001            |
|                     | <i>vps-34/lipI-4*</i>     | 15.7                            | 60/100           | 20.9                             | 72/100           | -25              | <0.0001            |

**Table S3.** Lifespan analysis of non-sterile *mes-1(bn17)* (WT), sterile *mes-1(bn17)* and *glp-1(e2141)* animals treated with different RNAi clones during adulthood. The table is organized so lifespan experiments carried out in parallel in WT and *mes-1* animals are in the same order. Data shown includes average of mean (avg. lifespan) lifespan, observed events (# of RNAi animals), % mean lifespan difference vs. control ( $\Delta$  avg) and *P* value vs. control. *P* values calculated with Log Rank (Mantel-Cox) test (\*survival curves shown in Figures S2I and S5F. For Fig. S2I, the experiment in which sterile *mes-1* animals showed the largest lifespan extension compared to WT was chosen. For Fig. S5F, a representative set was chosen randomly).

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Strains

We obtained the following strains for this study:

N2 Bristol, CF1903/*glp-1(e2141)*, CF1037/*daf-16(mu86)*, CF1880/*daf-16(mu86); glp-1(e2141)*, CF1041/*daf-2(e1370)* (from Kenyon lab), SB149/*mes-1(bn7)* (from *Caenorhabditis* Genetics Center), GC888/*glp-1(bn18)* (from Schedl lab via Hubbard lab), DA2123/*adls2122[lgg-1p::gfp::lgg-1 + rol-6]* (Kang et al., 2007), *mgEx[lipl-4p::lipl-4::sl2::gfp + myo-2p::mcherry]* (LIPL-4 OE, Wang et al., 2008), OP37/*unc-119(ed3)*; *Is[fosmid::pha-4::gfp::3xFLAG + unc-119]* (PHA-4 OE #1, Zhong et al., 2010), AGD84/*uthIs304[pha-4p::pha-4::gfp + rol-6]* (PHA-4 OE #2, Panowski et al., 2007).

We created the following new strains for this study:

MAH20/*daf-16(mu86); adls2122[lgg-1p::gfp::lgg-1 + rol-6]*,  
MAH44/*glp-1(e2141); adls2122[lgg-1p::gfp::lgg-1 + rol-6]*,  
MAH50/*daf-16(mu86); glp-1(e2141); adls2122[lgg-1p::gfp::lgg-1 + rol-6]*,  
MAH54/*adls2122[lgg-1p::gfp::lgg-1 + rol-6]; mgEx[lipl-4p::lipl-4::sl2::gfp + myo-2p::mcherry]*,  
MAH68/*mes-1(bn-7); adls2122[lgg-1p::gfp::lgg-1 + rol-6]*;  
MAH88/*glp-1(e2141); mgEx[lipl-4p::lipl-4::sl2::gfp + myo-2p::mcherry]*,  
QU10/*izEx5[lgg-1p::gfp::lgg-1 + odr-1p::rfp]*,  
QU11/*glp-1(e2141); izEx5[lgg-1p::gfp::lgg-1 + odr-1p::rfp]*,  
QU13/*glp-1(bn18); izEx5[lgg-1p::gfp::lgg-1 + odr-1p::rfp]*.

### Lifespan Analysis and RNAi Experiments

Eggs were raised on agar plates seeded with OP50 *E. coli*. Following development (or day 1 of adulthood), worms were transferred on plates seeded with HT115 *E. coli* bacteria expressing dsRNA for the corresponding target gene, and incubated at 20°C thereafter. Bacteria expressing specific dsRNA were obtained from the Ahringer (Kamath et al., 2003) and the Vidal libraries (Rual et al., 2004). Worms were usually counted every other day from day 1 of adulthood on a Zeiss dissection microscope. Censoring rate varied between 10 and 40%, which was primarily caused by worms crawling out of the plate or explosions (~10% of germline-less animals were censored from explosions). Autophagy RNAi clones (from either the Ahringer or the Vidal RNAi libraries) was confirmed by sequencing. The *tor* RNAi clone was from the Avruch lab (Long et al., 2002), and this RNAi clone decreased *tor* mRNA levels ~3-fold in both *glp-1* and in wild-type animals (data not shown). Moreover, this RNAi clone was specific to the *tor* locus, and did not affect mRNA levels of the Mit/Age gene B0261.6 (data not shown). We used adult-only RNAi of autophagy genes to avoid the developmental effects of blocking autophagy (Takacs-Vellai et al., 2005); these treatments generally reduced autophagy gene expression to about 30% of the original levels (data not shown), which might be sufficient to sustain basal levels of autophagy in wild-type animals.

### Autophagic Detection by Electron Microscopy

For electron microscopic analysis of autophagy, N2 wild-type or *glp-1(e2141)* eggs were incubated for 2 days at 25°C, collected and prepared for analysis according to Troemel et al, 2008 (except we omitted the step of preparing animals with a razor blade).

### Autophagy Quantification by GFP::LGG-1 reporter

Autophagic events were observed using a fluorescent microscope Zeiss Imager Z1. *C. elegans* expressing GFP::LGG-1 were placed on 2% agarose pads and foci were counted (using 1000-fold magnification on a Zeiss Axioplan II microscope) in the seam cells of L3 larvae and day 1 adult worms. Similarly, autophagy was measured in the intestinal cells of different worms by counting foci in the proximal region of the intestine, encompassing the first three intestinal cells starting beyond the pharyngeal grinder. To corroborate findings in *glp-1(e2141)*; *adls2122[lgg-1p::lgg-1::gfp + rol-6]* animals, a strain expressing GFP::LGG-1 carrying another *glp-1* loss-of-function allele, *glp-1(bn18)*, was created by independent injection of the *lgg-1* reporter construct (as in Melendez et al., 2003) along with an *odr-1p::rfp* co-injection marker. For all experiments, 2-3 independent biological repeats were carried out and the mean number of foci was averaged and plotted.

### Real-time Quantitative PCR

RNA was extracted from flash frozen worm pellets (in biological triplicates) using TRIZOL reagent. Extracts were cleaned with RNeasy QIAGEN kits. The M-MuLV reverse transcriptase (NEB) and oligo-dT primers were used for reverse transcription of 1 µg of RNA per samples. A Roche 480 LightCycler and SYBR Green Master Mix (Roche) was used for RT-PCR analyses. Quantification was performed by conversion of observed  $C_t$  values to relative values using a standard curve for each target gene. The attached software, Roche LightCycler 480 SW 1.5, was used to calculate relative values. Target genes were normalized with values obtained by RT-PCR of the housekeeping gene cyclophilin (*cyn-1*). Similar results were obtained by normalizing with other housekeeping genes (*nhr-23* and *ama-1*). Primer sequences and conditions are available upon request.

### Immunoblotting

Worms (~500-1000) were collected at day 1 of adulthood with M9 buffer and pelleted by gravity. Pellets were flash frozen in liquid nitrogen and resuspended in 100 µl of 0.2% SDS-RIPA lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100). Samples were then sonicated and boiled for 10 minutes. Insoluble proteins were pelleted at high speed in a microcentrifuge for 5 minutes and supernatants were collected. Protein concentration was determined using the detergent-compatible BCA assay (Bio-Rad). For gel electrophoresis, 50 µg of protein was loaded in each well of a 4-12% Bis-Tris gel (Invitrogen). Resolved proteins were then transferred onto a nitrocellulose membrane, which was subsequently blocked and incubated with Rabbit anti-TOR antibody (Sigma, T2949) and Goat anti-tubulin antibody (Santa Cruz, SC12836). The anti-TOR antibody likely detected *C. elegans* TOR as *tor* RNAi reduced the intensity of a ~280 kDa band (data not shown).

### LIPL-4 Expression

The expression pattern of LIPL-4/K08A4.5 in adult worms was evaluated in transgenic animals of the strains *mgEx[lipl-4p::lipl-4::sl2::gfp + myo-2p::mCherry]* (LIPL-4 OE) (Wang et al., 2008) and *MAH84[glp-1(e2141); mgEx[lipl-4p::lipl-4::sl2::gfp + myo-2p::mcherry]* (which was generated by crossing the LIPL-4 OE strain to



CF1903/*glp-1(e2141)*), and micrographs were acquired on a Zeiss Axioplan II microscope equipped with a Zeiss Apotome using 1000-fold magnification.

### **Autophagy Gene Promoter Analysis**

To assess the frequency with which PHA-4/FOXA binds to autophagy promoters, we analyzed datasets from two recent publications (Niu et al., 2011, and Zhong et al., 2010), which functionally investigated PHA-4 binding to promoters during development and during larval starvation. We found that PHA-4 can bind to several promoters in addition to *unc-51*, *lgg-1*, and *bec-1* can bind PHA-4, at least during development. The autophagy genes include *atg-2*, *atg-13*, *atg-14*, and *atg-16*, whereas others that are not bound by PHA-4 include *atg-4*, *atg-5*, *atg-12*, and *vps-34* (data not shown). These data suggest that PHA-4 could regulate many, but not all, autophagy genes in *C. elegans*. However, it is not clear from these binding analyses how many of these genes will be *bona fide* transcriptional targets in the adult animals.

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